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Effect of maturity and cold storage on ethylene biosynthesis and ripening in 'Bartlett' pears treated after harvest with 1-MCP

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ABSTRACT

To further our understanding of the response of 'Bartlett' pear fruit to 1-methylcyclopropene (1-MCP) and their ability to recover the capacity to ripen, 'Bartlett' pears were treated with 0.3 $\mu L\,L^{-1}$ 1-MCP for 12 h at 20 °C immediately after harvest in two seasons and to pear fruit of four maturities. 1-MCP decreased rates of softening, ethylene production, respiration, and yellow color development, and reduced incidence of scald and internal breakdown. Ripening recovery induced by cold storage of 1-MCP treated fruit depended on maturity and season and was associated with stimulated ethylene production, including 1-aminocyclopropene carboxylic acid synthase (ACS) activity, 1-aminocyclopropene carboxylic acid oxidase (ACO) activity, and transcript levels of genes associated with these enzymes.

1. Introduction

1-MCP is an ethylene action inhibitor that has been used after harvest to retard ripening in a range of fruits (Sisler and Blankenship, 1996; Sisler and Serek, 2003; Watkins, 2006). In pear fruit, 1-MCP treatment has been reported to decrease softening, color development, respiration rates, and ethylene production (Baritelle et al., 2001; Argenta et al., 2003; Kubo et al., 2003; Hiwasa et al., 2003; Calvo and Sozzi, 2004, 2009; Calvo, 2004; Ekman et al., 2004; Trinchero et al., 2004; Mwaniki et al., 2005).

California 'Bartlett' pears are normally harvested in July and August and cool-stored until they are marketed, generally within 60–90 d to avoid losses due to the development of superficial and senescent scald and internal breakdown (California Pear Advisory Board, personal communication). Ethylene produced by the fruit during storage can exacerbate the incidence of these physiological disorders, and 1-MCP is highly effective to control or reduce their incidence (Du and Bramlage, 1994; Watkins et al., 1995; Whitaker and Solomos, 1997; Ju and Curry, 2000; Bower et al., 2003; Ekman et al., 2004; Gapper et al., 2006).

Although postharvest application of 1-MCP provides valuable benefits, it is challenging to obtain normal softening and ripening in 1-MCP-treated California 'Bartlett' pears if the treatment is performed immediately after harvest (Ekman et al., 2004; Mitcham, unpublished). The 1-MCP treatment appears to reinforce the natural characteristics of European pears which are resistant to ripening after harvest, and require a period of cold storage or ethylene exposure to induce ripening (Villalobos-Acuña and Mitcham, 2008).

Cold storage induces synthesis of the enzymes involved in ethylene biosynthesis: ACC synthase (ACS) and ACC oxidase (ACO) (Sfakiotakis and Dilley, 1974; Chen et al., 1983; Knee, 1987; Blankenship and Richardson, 1985). Genes encoding putative ACS and ACO sequences have been isolated from pears (Lelièvre et al., 1997; Pech et al., 2002; El-Sharkawy et al., 2004; Fonseca et al., 2005; Kondo et al., 2006), but their transcript levels and the identity of those playing a major role during ripening in 1-MCP treated 'Bartlett' pears have not yet been described.

Fruit maturity and growing region have an effect in the ripening behavior of pears even after ethylene or cold storage treatments (Agar et al., 1999) and likely can affect 1-MCP response as well. In this study, we test the hypothesis that the induction of ripening during cold storage of 1-MCP-treated pears might be greater in pears harvested at more mature stages.

2. Material and methods

'Bartlett' pears were harvested at harvest maturities of 91 and 81 N flesh firmness (early and normal harvest maturities, respectively) on July 29 and August 5, 2005 from a commercial orchard in Ukiah, CA. In 2006, pears were obtained directly from Alex R.

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Thomas & Co. packinghouse in the same area at 76 and 69 N flesh firmness (mid-harvest and late-harvest, respectively) on August 14 and 21. Fruit were held overnight at 20 °C, sorted to remove damaged or blemished fruit and randomized. Half of the fruit were loaded into perforated plastic field lugs and placed in a sealed plastic tent fitted with a circulation fan, and exposed to 0.3 μL L^{-1} 1-MCP at 20 °C for 12 h released from Smart Fresh® tabs provided by AgroFresh® (Springhouse, PA) while fruit of the other half were held as untreated control fruit at the same temperature. A subset of 1-MCP-treated fruit and control fruit were treated after the 1-MCP application with 100 μL L^{-1} of ethylene for 24 h at 20 °C then held at 20 °C in air, a standard treatment for fruit marketed immediately after harvest. The remaining fruit were placed into 16 kg vented cardboard pear boxes for cool storage at −1 °C for 120 (2005), 105 (2006) and 180 d (both seasons). Plastic liners with 2.5 cm diameter vent holes allowing gas exchange were used in 2006 to reduce water loss during storage. Fruit were evaluated for skin color, firmness, CO_2 and ethylene production, defects, and overall quality upon removal from storage and after ripening.

2.1. Fruit evaluation

Fruit was evaluated immediately after each storage time and after ripening at 20 °C. Ripening time varied depending on storage duration, and was determined when untreated fruit were fully ripe (10–18 N firmness). Eight fruit per replication (a total of 24 for each treatment in 2005 and 32 in 2006) were assessed for color and firmness at each evaluation time. In addition, 6 fruit per replication were used to determine ethylene and CO_2 production at 20 °C during ripening.

Firmness was measured objectively on two sides of each fruit after a thin slice of skin was removed using a Fruit Texture Analyzer (Gűss, Strand, Western Cape, South Africa) fitted with an 8-mm probe. Carbon dioxide and ethylene production at 20 °C was measured by placing 6 fruit from each block or repetition into a 3.8 L jar and sealing it for 10–60 min, depending on the respiration rate. The headspace gas was evaluated for the concentration of CO_2 using rapid gas analysis (VIA510, Horiba, Fukuoka, Japan) and ethylene using flame ionization gas chromatography (Model 211 Series S, Hach-Carle Co., Fullerton, CA) utilizing two columns (1.22 m and 0.305 m, 8% NaCl on Alumina F-1 80/100 DV, Chandler Engineering – Carle Chromatography, Tulsa, Oklahoma). Nitrogen was used as the carrier gas at a flow rate 30 mL/min, and the injector port, detector port and oven temperatures were at 80 °C. A 10 mL headspace sample was injected into a 2 mL fixed sample volume valve. Color was rated subjectively using the California Department of Food and Agriculture color chart (1 = green; 2 = light green; 3 = light yellow; 4 = yellow, Calif. Dept. Food and Agric., Sacramento, CA). Internal browning and scald (both superficial and senescent) incidence were calculated as the percentage of the total evaluated fruit affected by each disorder.

2.2. Enzyme analysis

2.2.1. In vitro ACS activity

ACS activity was determined similarly to the method described by Dandekar et al. (2004). Skin tissue was frozen in liquid N_2 and stored at −80 °C until use. Tissue (5 g) was homogenized in a Polytron (Kinematica AG Littau, Switzerland) with 2 L kg^{-1} of 4 °C extraction buffer [400 mM potassium-phosphate (pH 8.5), 1 mM EDTA, 0.5% 2-mercaptoethanol, 0.01 mM pyridoxal phosphate]. The homogenized tissue was filtered through two layers of cheesecloth and centrifuged at 28,000 × *g* for 30 min. The pellet was re-suspended in the same amount of extraction buffer and centrifuged for 20 min as described previously. After centrifugation, 5 mL of solubilization buffer [20 mM potassium-phosphate (pH 8.5), 1 mM EDTA, 1 mM 2-mercaptoethanol, 10 μM pyridoxal phosphate, 30% (v/v) glycerol and 1% (v/v) Triton X-100] as described by Yip et al. (1991) was added to the pellet which subsequently was homogenized with a Polytron.

The ACS assay was as described by Lizada and Yang (1979) and Yip et al. (1991) in 15 mL test tubes, with each experimental unit assayed in two separate tubes; one containing the 'sample' and other the 'internal standard' (IS). The 'sample' tube contained 100 μL of the solubilized pellet described above, 400 μL of freshly prepared assay buffer (10 mL 500 μM adenosyl methionine + 10 mL 100 mM HEPES & 20 μM pyridoxal phosphate) and 300 μL deionized water. The IS tube had the same amount of homogenized pellet and assay buffer as the 'sample' tube, but 50 μL of 1-aminocyclopropene carboxylic acid (ACC) and 250 μL deionized water. Test tubes were capped and incubated for 2 h at 30 °C, after which 100 μL 10 mM $HgCl_2$ was added. Rubber stoppers were placed on the top of the tube and approximately 100 μL of a solution containing 2:1 5% NaOCl:saturated NaOH was added using a fine needle. Tubes were vortexed, kept on ice for at least 3 min and the ethylene concentration in a 1 mL headscape sample was determined by gas chromatography.

2.2.2. In vitro ACO assay

Skin tissue was frozen in liquid N_2 and stored at −80 °C until use. ACO activity was determined using 5 g fresh tissue as described by Agar et al. (1999) and Dandekar et al. (2004).

2.2.3. Relative ACS and ACO activity

Enzyme levels of the untreated fruit at harvest were assigned a relative activity of 1.0. The remaining values after different storage times were calculated in relation to this initial value, showing the relative increase or decrease.

2.3. Gene expression

Transcript levels of genes associated with ethylene biosynthesis were determined in tissues from fruit that had the highest ripening induction after treatment with 1-MCP and cold storage: 69 N firmness at application in 2006. RNA isolation and cDNA synthesis was performed according to the method of MacLean et al. (2007). Approximately 2–3 g of frozen tissue was ground to a fine powder with a mortar and pestle prechilled with liquid N_2. Total RNA was extracted using the Qiagen RNeasy Plant Mini Kit®, using the supplied RLT extraction buffer, according to the instructions in the kit. RNA was eluted from the column with 30 μL sterile water and quantity and quality was evaluated using a DU 800 spectrophotometer (Beckman Coulter, Fullerton, CA). First-strand cDNA synthesis was performed using Invitrogen's Superscript™ III First Strand Synthesis Systems for RT-PCR Systems using oligo (dT) and ramdom hexamer as primers. For the cDNA synthesis reaction ≈ 3 μg RNA, 1 μL 50 μM oligo (dT), 1 μL 50 ng random hexamer, 1 μL 10 mM dNTP mix, and sterile water to 10 μL were mixed and heated at 65 °C for 5 min to denature the RNA. After placing the reaction mixture on ice, the remaining components were added; 2 μL 10X RT Buffer, 4 μL 25 mM $MgCl_2$, 2 μL 0.1 M DTT, 1 μL RNase Out (40 u/μL), and SSIII RT (200 u/μL) reverse transcriptase enzyme. The 20 μL reaction mixture was then incubated for 10 min at 25 °C, 50 min at 50 °C and 5 min at 85 °C. The reaction mixture was subsequently chilled on ice while 1 μL RNAse H was added to each tube and then incubated 20 min at 37 °C. The cDNA was either stored at −80 °C or used immediately for quantitative reverse transcriptase polymerase chain reaction.

cDNA was used as a template to perform quantitative RT-PCR (RT-qPCR) analysis using 7300 Real Time PCR System Applied Biosystems and SYBR Green PCR Master Mix (Applied Biosystems, Carlsbad, CA). The primers for *PcACO1* were designed based on

Table 1
Primers used for RT-qPCR analysis.

Name	Oligonucleotide sequence	Size of PCR product (bp)	Reference
18S rRNA	F 5′CATGGCCGTTCTTAGTTGGTGGAG-3′ R 5′AAGAAGCTGGCCGCGAAGGGATAC-3′	110	Chen et al. (2004)
PcACS1	F 5′-TCCTTTCCACACTTCTGTCTTACAGC-3′ R 5′-GGGTAGGAATAAGAAGAACTTCGCCGG-3′	483	El-Sharkawy et al. (2004)
PcACS2b	F 5′-CTTTCTTGGAGGCCA AAGTGCTTT-3′ R 5′GACGAAAGTCAATAGCTCATTTGCTGC-3′	482	El-Sharkawy et al. (2004)
PcACS4	F 5′CTTGGTTGAAGAGTGGATTAG-3′ R 5′ ATGATCAAGCCCTTGACATTG-3′	432	
PcACS5	F 5′TTTCGACACAAACTCAGCATCT-3′ R 5′-AAAGCAACTTCCATGGTCTTGT-3′	352	
PcACO	5′AATGCACCACTCCATTGTCATA-3′ 5′GCTTCATGTAGTCATCAAACACA-3′	236	Fonseca et al. (2005)

the previously published *PcACO1* from *Pyrus communis* [AJ504857 (Fonseca et al., 2005)]. *PcACS1a* (X87112), *PcACS2b* (AY388989), *PcACS4* (AF386518), and *PcACS5* (AF386523) primers were designed according with previous work from El-Sharkawy et al. (2004) with modifications for *PcACS4* and *PcACS5* as described in Table 1. The abundance of 18S rRNA (Chen et al., 2004) was used as an internal control. Every reaction contained 1–4 μL cDNA, 12.5 μL SYBR Green, 1 μL of each forward and reverse primers, and water to a total volume of 25 μL. Data were analyzed using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) and is presented as the relative level of gene expression.

Parallel to the RT-qPCR analysis, PCR for all clones were performed using the My Cycler Thermal Cycler Model No. 580BR5895 (Bio-Rad, Hercules, CA). An aliquot of the PCR reaction was loaded onto a 1% (w/v) agarose gel (110 V) to confirm a single product amplified in the reaction. The remaining PCR product was cleaned using the QIAquick® PCR Purification Kit to remove any potential contaminants, cloned using the P-Gem-T-Easy Vector System (Promega) and sequences revised in the NCBI database (http://www.ncbi.nlm.nih.gov/).

2.4. Experimental design and data analysis

The experimental design was a randomized complete block with three blocks total in 2005 and a completely randomized design with four replications in 2006. Data were analyzed using SAS (SAS Institute, Cary, NC) and log transformation and/or Winsorization were performed in some data sets to fulfill ANOVA assumptions as indicated in figure legends. In 2006, the experiment was set up using a completely randomized design, but because of systematic temporal variation among the four replicates analyzed for gene expression for each treatment, the data were analyzed as a randomized complete block, reflecting the temporal variability among the replicates. Regressions were carried out using SigmaPlot for Windows version 10.0 (Systat Software, Inc. Chicago, IL).

3. Results

3.1. Changes during ripening

1-MCP treatment at 0.3 μL L^{-1} immediately after harvest delayed softening rates (Fig. 1), and decreased ethylene production (Fig. 2) during ripening soon after harvest and ripening after all cold storage periods. Only the fruit harvested at 69 N flesh firmness in 2006 softened normally after 180 d at −1 °C, softening to a ready to eat condition with softening rates similar to the untreated fruit (Fig. 1), but without producing similar levels of ethylene (Fig. 2) and respiration rates (data not shown). Yellow skin color development was delayed, especially at harvest and after 105–120 d storage, but not after 180 d storage at −1 °C (Fig. 3).

3.2. Effect of 1-MCP on scald and internal breakdown (IB)

1-MCP treatment eliminated or reduced scald and IB incidence and severity in both harvest seasons and all maturities tested (Figs. 4 and 5). In 2006, the most mature fruit treated with 1-MCP

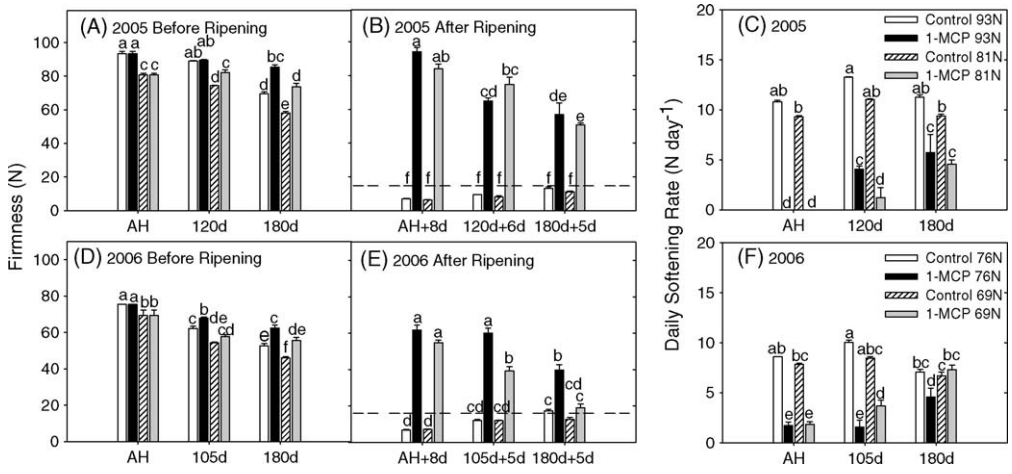

Fig. 1. Firmness before (A and D) and after ripening at 20 °C (B and E), and daily softening rates (C and F) of untreated and 1-MCP treated fruit in 2005 (A–C) and 2006 (D–F) as determined at harvest and after 120 (2005) or 105 d (2006) and 180 d (both years). Fruit were treated at harvest (AH) at two maturities each year, 93 and 81 N in 2005 and 76 and 69 N in 2006. Different letters within each figure indicate means that are statistically different (Tukey, $\alpha \leq 0.05$) using Winsorization or transformed data to fulfill ANOVA assumptions. Horizontal dashed lines mark firmness of fruit considered ready to eat.

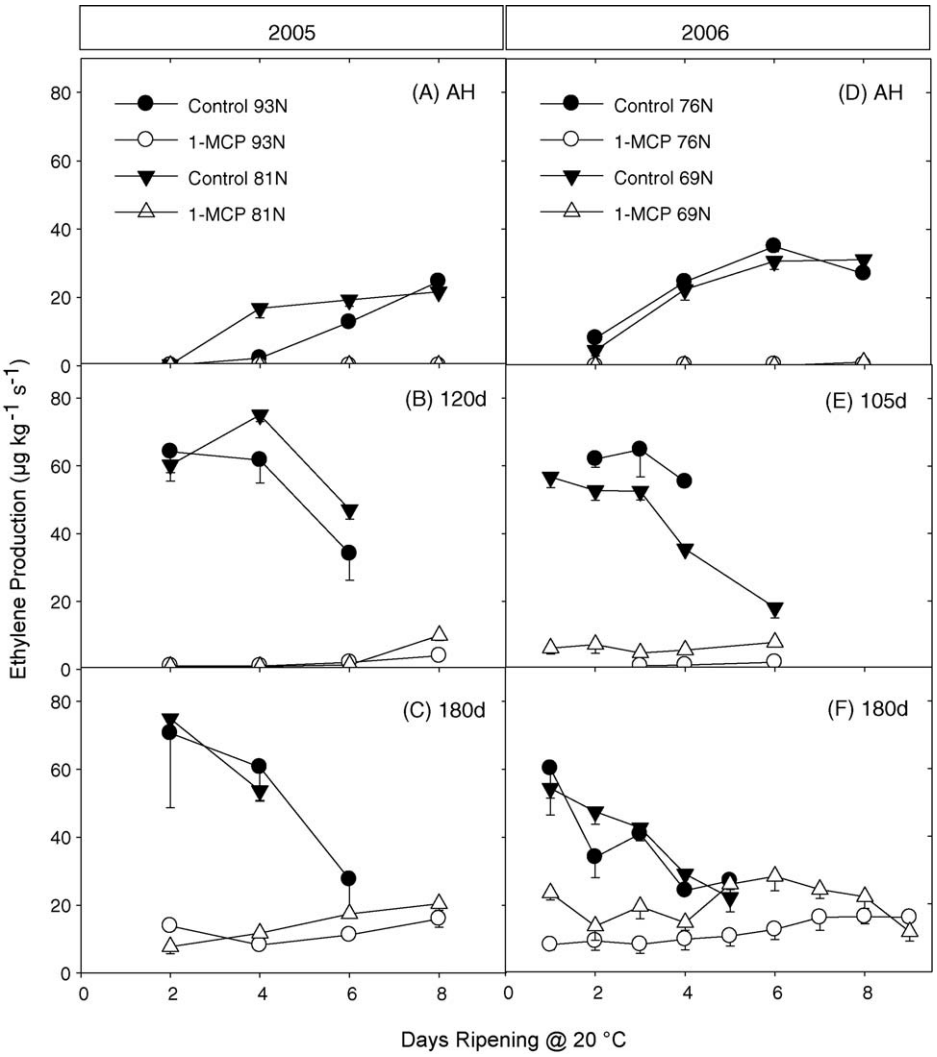

Fig. 2. Ethylene production during ripening in untreated and 1-MCP-treated fruit in 2005 (A–C) and 2006 (D–F). Fruit were treated at harvest at two maturities each year, 93 and 81 N in 2005 and 76 and 69 N in 2006 and were ripened at harvest (AH) and after 120 (2005) or 105 d (2006) and 180 d (both years). Vertical lines represent the standard error of the mean.

had some scald incidence after 180 d cold storage at −1 °C and subsequent ripening (Fig. 4B), but the severity of these symptoms was very mild compared with those on the untreated fruit (data not shown). In 2005, IB was not detected, but this physiological disorder was controlled completely by 1-MCP in 2006 (Fig. 5).

3.3. *Effect of cold storage on ACS and ACO activities and transcript levels*

ACS and ACO activities were determined on tissue from treated and control fruit at both maturities. ACS and ACO activity in both treatments increased during cold storage, but the increase was greater in the untreated than in the 1-MCP treated fruit. Even in the most mature fruit stored for 180 d, activity of both enzymes in the 1-MCP-treated fruit was less than a third of that in the controls (Fig. 6).

The expression of *PcACO*, *PcACS1* in 1-MCP treated-fruit and *PcACS4* and *PcACS5* in the untreated and 1-MCP-treated fruit was moderately induced by cold storage; with transcripts for *PcACO* and *PcACS1* showing the highest expression levels (Fig. 7). However, transcript levels of *PcACO*, *PcACS4*, and *PcACS5* were lower in 1-MCP-treated fruit than those in control fruit after 105 d storage at −1 °C, but not after 180 d at −1 °C; when this fruit recovered the ability to soften (Fig. 1E and F) and produce ethylene (Fig. 2F). In contrast, *PcACS2b* transcript levels were reduced during cold storage, with the highest levels in the untreated fruit at harvest (data not shown).

An analysis was carried out to determine whether ACS and ACO gene expression exhibited comparable relative changes during cold storage as observed for ACS and ACO enzyme activity (Fig. 8). Untreated and 1-MCP-treated fruit had similar relative ACS activity as relative total *PcACS* (cumulative *PcACS1*, *PcACS2b*, *PcACS4*, *PcACS5*) gene expression except for the untreated fruit after 180 d cold storage in which total *PcACS's* gene expression had significantly lower relative changes than ACS activity (Fig. 8A). In both untreated and 1-MCP-treated pears, ACO activity had higher relative changes than those obtained with *PcACO* gene expression during all evaluation times except for the untreated fruit at harvest and 1-MCP-treated fruit after 180 d cold storage for which no significant differences were found (Fig. 8C and D).

To characterize whether ACS and ACO enzyme activity correlated with gene expression levels, each pair of values from individual replications was plotted and regression analysis carried out separately for untreated and 1-MCP-treated fruit. For both ACS and ACO, only the 1-MCP-treated fruit showed a quadratic trend (Fig. 9A–D).

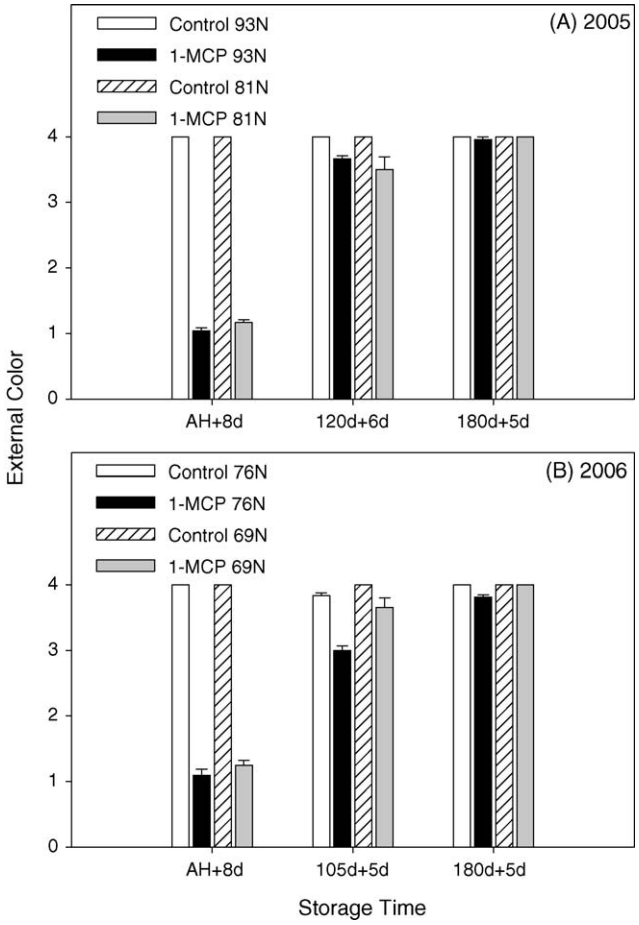

Fig. 3. External color after ripening of untreated and 1-MCP-treated fruit in 2005 and 2006. Fruit were treated at harvest at two maturities each year, 93 and 81 N in 2005 and 76 and 69 N in 2006. Fruit were ripened at harvest (AH), after 120 (2005) or 105 d (2006) and 180 d (both years) at 20 °C for the days indicated in *x*-axis labels. Color was subjectively graded according to the California Department of Agriculture Bartlett pear color chart: 1 = green; 2 = light green; 3 = light yellow; 4 = yellow. Vertical lines represent the standard error of the mean.

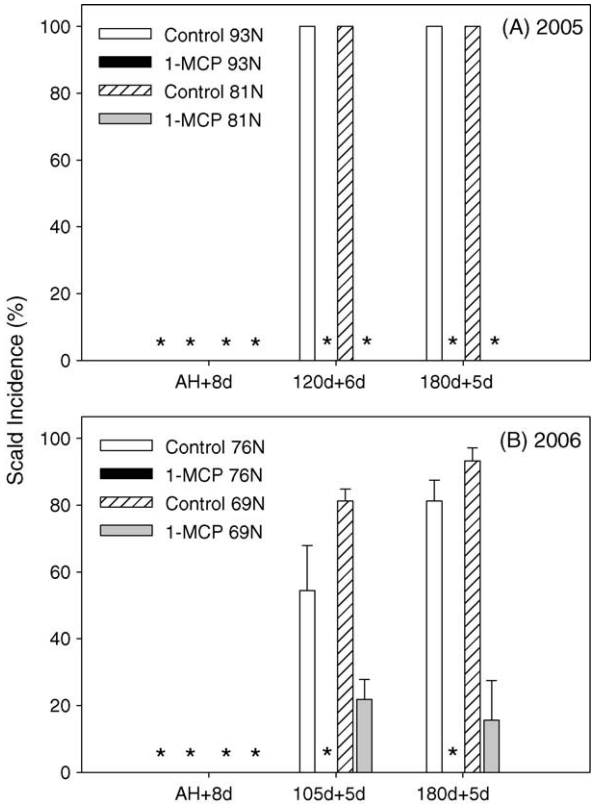

Fig. 4. Scald incidence (%) after ripening of untreated and 1-MCP-treated fruit in 2005 and 2006. Fruit were treated at harvest at two maturities each year, 93 and 81 N in 2005 and 76 and 69 N in 2006 and ripened at harvest, after 120 (2005) or 105 d (2006) and after 180 d cold storage (both years) at 20 °C for the days indicated in *x*-axis labels. Vertical lines represent the standard error of the mean. * = not detected.

4. Discussion

In this study, we hypothesized that the induction of ripening during cold storage of 1-MCP-treated pears might be greater in pears harvested at higher maturities. However, only during one season more mature fruit slightly to moderately recovered ripening capacity after being stored at −1 °C. This suggests that maturity based on fruit firmness might not be an appropriate indicator for reliably predicting the strength of the response of 'Bartlett' pear fruit to 1-MCP.

The differences in ethylene production and recovery of softening rates, especially in 2006, in fruit that were more mature versus less mature at the time of treatment might be associated with the abundance of ethylene receptors at the time of the 1-MCP treatment. In this study, fruit were exposed to 1-MCP for 12 h which produced strong ripening inhibition in both seasons. Since ethylene receptor proteins negatively regulate ethylene response, high abundance of these proteins at the time of the 1-MCP treatment might induce stronger 1-MCP responses.

Cold storage increased ethylene production both in untreated and 1-MCP-treated fruit (Fig. 2), but the induction of higher softening rates during ripening by cold storage was statistically significant only in 1-MCP treated fruit (Fig. 1C and F). The increase in ethylene production and partial to moderate recovery of ripening in 1-MCP-treated fruit paralleled the observed increase in ACS and ACO activity (Fig. 6) and the induction of transcription in some of the genes encoding these enzymes (Fig. 7).

The fact that the relative total *PcACS's* expression levels (Fig. 8A and B), especially for the 1-MCP-treated fruit, were statistically similar to the relative changes in ACS activity suggests that transcription of these genes might play an important regulatory role in ACS activity. To further characterize this, a regression analysis for *PcACS* gene expression and ACS enzyme activity was carried out

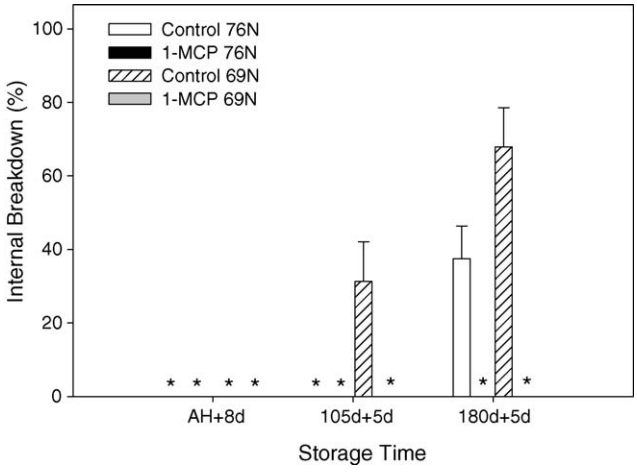

Fig. 5. Internal breakdown incidence (%) after ripening in untreated and 1-MCP-treated fruit. Fruit were harvested at 76 and 69 N in 2006 and ripened at harvest, after 105 and 180 d of cold storage at 20 °C for the days indicated in the *x*-axis labels. Vertical lines represent the standard error of the mean. * = not detected.

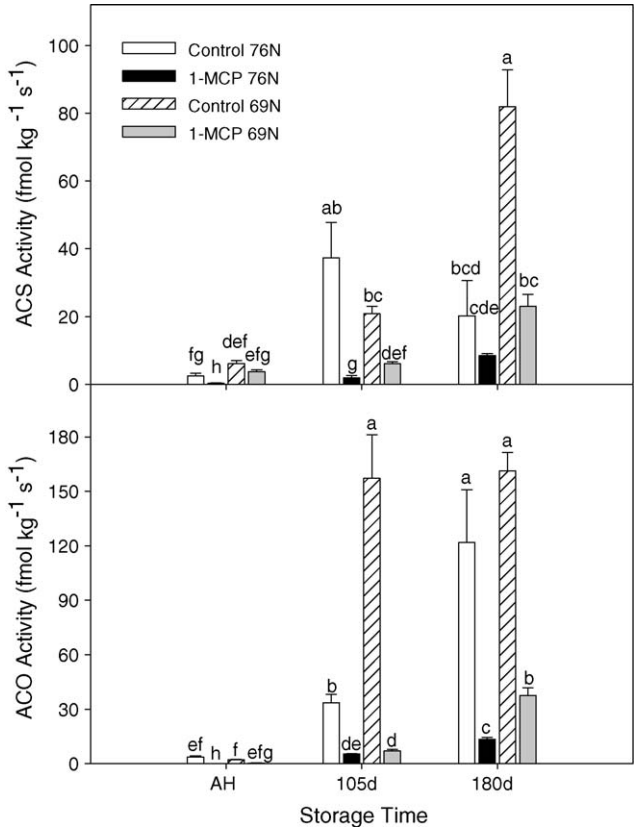

Fig. 6. *In vitro* ACC synthase (ACS) and ACC oxidase (ACO) activity of untreated and 1-MCP-treated fruit measured at harvest and immediately after 105 and 180 d of cold storage. Fruit were harvested and immediately treated at 76 and 69 N maturity in 2006. Vertical lines represent the standard error of the mean. Mean separation with Tukey ($\alpha \leq 0.05$) using transformed data to fulfill ANOVA assumptions. Means with different letters are significantly different.

among all the repetition values obtained during the distinct storage intervals (Fig. 9A and B). This analysis revealed a quadratic relationship in the data obtained for the 1-MCP-treated fruit (Fig. 9A), but not for the untreated fruit (Fig. 9B). The regression shows that an important proportion ($R^2 = 0.85$) of the variability in enzyme activity correlated with the variability in the *PcACS* gene expression data. It is unknown why a similar trend was not found in the untreated fruit (Fig. 9A) even thought the relative changes in both *PcACS* gene expression and enzyme activity were statistically similar at harvest and after 105 d cold storage (Fig. 8A). Besides transcriptional control, other regulatory mechanisms such as ACS protein stability have been shown to play an important role in some developmental processes in plants (Chae et al., 2003; Argueso et al., 2007) and our data suggests that it is likely that untreated and 1-MCP-treated fruit had distinct mechanisms in place to regulate ACS activity.

Relative ACO enzyme activity was significantly different from *PcACO* expression levels in untreated and 1-MCP-treated fruit at all storage times except at harvest for the untreated fruit and after 180 d cold storage in the 1-MCP-treated fruit (Fig. 8C and D). This suggests that other regulatory mechanisms than the transcription of *PcACO* might regulate ACO activity. To further investigate, a regression analysis was made among all the repetition values obtained at the distinct storage times. As was the case for ACS, only the 1-MCP treated-fruit showed a significant correlation ($R^2 = 0.77$, Fig. 9D), indicating that the transcription of *PcACO* correlated to some extent with ACO enzyme activity. Since we studied only one gene encoding ACO, it is likely other genes or other regulatory mechanisms might play an important role in regulating ACO activity, especially for the untreated fruit in which we could not find good correlation between ACO activity and *PcACO* gene expression (Fig. 9C). The fact that we obtained a significant relationship in the 1-MCP-treated fruit (Fig. 9D) might suggest that *PcACO* plays an important role regulating the activity of ACO. Overall, our data also suggest that *PcACO* plays a distinct role regulating ACO activity in untreated and 1-MCP treated fruit (Fig. 9C and D).

Previous reports by Pech et al. (2002) and El-Sharkawy et al. (2004) found that transcription of *PcACS1a* increased particularly

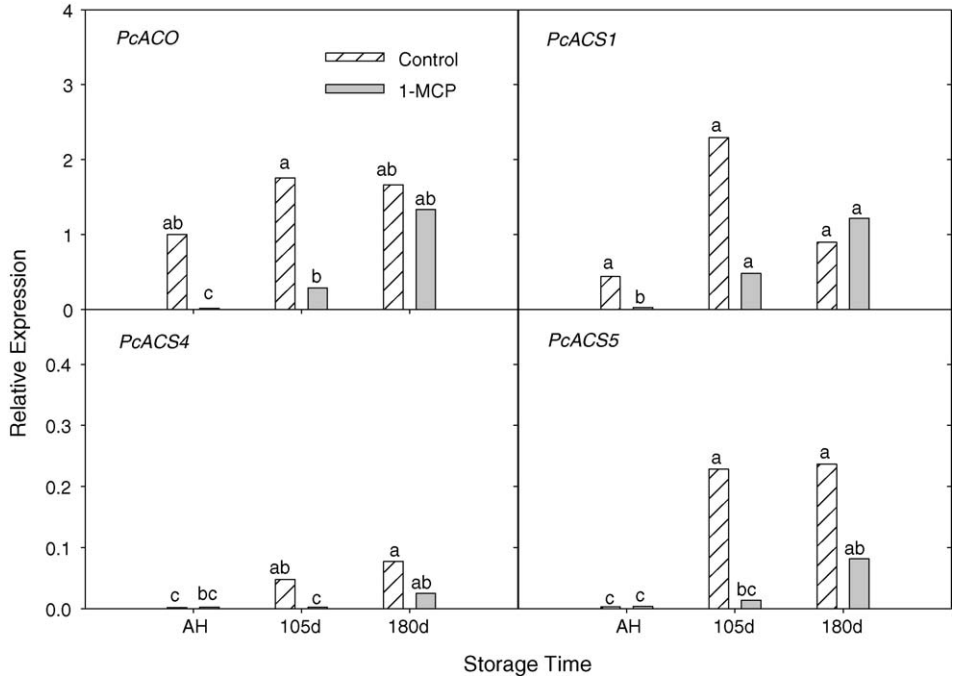

Fig. 7. Transcript levels of *PcACO1*, *PcACS1a*, *PcACS2b*, *PcACS4*, and *PcACS5* in untreated and 1-MCP treated fruit measured immediately at harvest and after 105 and 180 d of storage at −1 °C in 2006. Fruit were harvested at 69 N. Mean separation with Tukey using winzorization of transformed data to fulfill ANOVA assumptions. Means with distinct letters are significantly different (p value < 0.05).

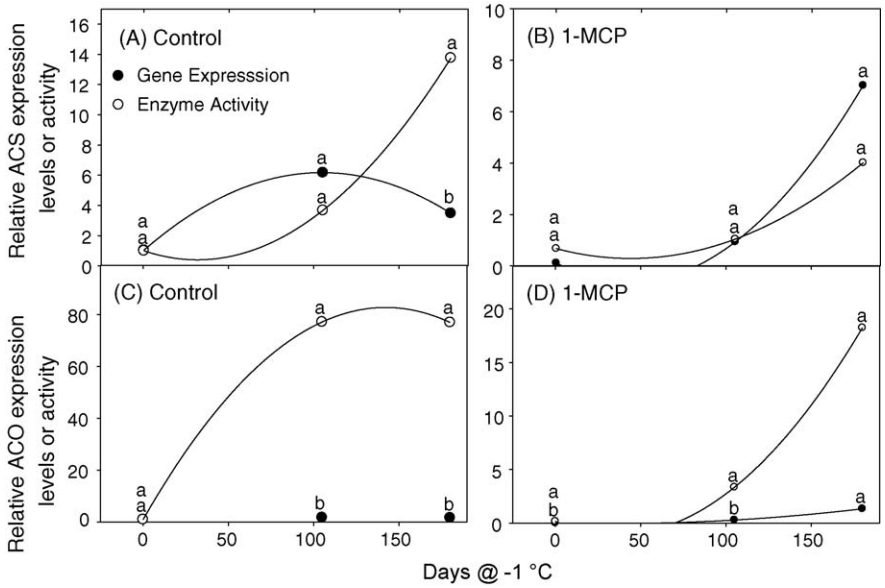

Fig. 8. Relative ACS activity, total *PcACS* (cumulative *PcACS1*, *PcACS2b*, *PcACS4*, and *PcACS5*) (A and B), Relative ACO activity and relative *PcACO* gene expression (C and D) in untreated and 1-MCP treated fruit measured immediately at harvest and after 105 and 180 d of storage at −1 °C in 2006. Fruit were harvested at 69 N. Mean separation with Tukey using regular or transformed data to fulfill ANOVA assumptions. Means with distinct letters are significantly different (p value < 0.05).

during 80 d of storage at 0 °C in 'Passe-Crassane' pears. In our study, we confirmed that *PcACS1* and *PcACO* were highly stimulated during cold storage particularly in 1-MCP-treated fruit (Fig. 7). The difference in transcript levels of 1-MCP treated and untreated fruit were significant at harvest and after 105 d storage at −1 °C, but it became less significant after 180 d storage at −1 °C. This same trend was not found for *in vitro* activity of ACO and ACS. Interestingly, in this study *PcACS2b* was not induced by cold storage (data not shown). El-Sharkawy et al. (2004) detected *PcACS2b* only during ripening of cold independent genotypes. Our analysis confirmed this gene presented much higher transcript levels when fruit were transferred to 10 or 20 °C for ripening (data not shown).

Studies by Kevany et al. (2007, 2008) and Chen et al. (2007) showed that ethylene exposure degrades two ethylene receptor proteins in tomato and one in Arabidopsis. Conversely, Kevany et al. (2007, 2008) showed that the degradation of ethylene receptor

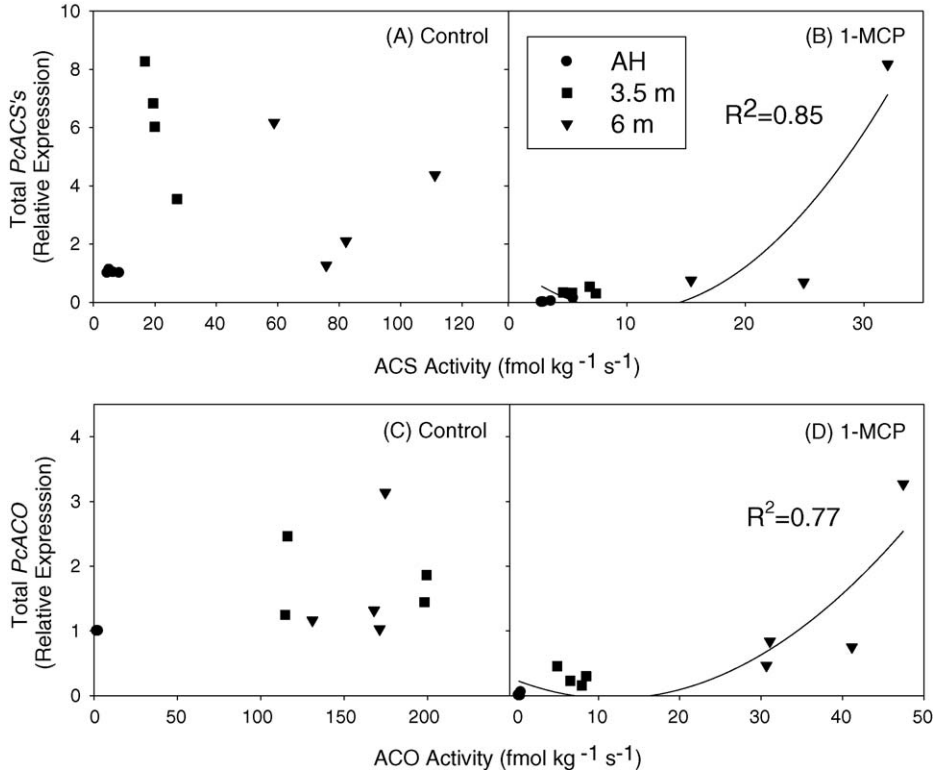

Fig. 9. Relationship between ACS activity and total *PcACS* (cumulative *PcACS1*, *PcACS2b*, *PcACS4*, and *PcACS5*) (A and B) or ACO activity and *PcACO* relative gene expression (C and D) in untreated and 1-MCP-treated fruit measured immediately at harvest and after 105 and 180 d storage at −1 °C in 2006. Fruit were harvested at 69 N.

proteins played a role in the development of ripening competence in tomatoes and that 1-MCP stabilized these two ethylene receptor proteins, so that they were no longer degraded by ethylene. It is not known whether this occurs in pears, but we showed in this study that the same 1-MCP concentration and treatment conditions caused diverse responses between pears of different maturity stages and cold storage periods. Whether these differences were caused by the abundance of ethylene receptors interacting with 1-MCP at the moment of the treatment and/or ethylene receptors turnover during cold storage is the subject of our ongoing research.

Other possibilities exist associated with ethylene receptor proteins interacting permanently with 1-MCP as has been suggested previously (Blankenship and Dole, 2003), and therefore the recovery of ripening capacity is produced by other proteins involved in the ethylene perception pathway or a combination of this and changes in ethylene receptors turnover during cold storage. The system involved in plant response to ethylene has ample plasticity; thus, the abundance of these proteins involved in ethylene action can change after 1-MCP treatment, changing the strength of the signaling. For example, *AtEIN3* and *AtEIL1*, transcription factors located downstream of ethylene perception, have been suggested to be essential for rapid and highly sensitive responses to environmental stresses or plant development (Kendrick and Chang, 2008); and changes in transcription and protein levels of these two transcription factors may be promoted by long exposure to low temperatures during cold storage.

Overall, 1-MCP provided a very desirable reduction in the incidence of scald and internal breakdown in California 'Bartlett' pears. These two physiological disorders limit the commercial storage life of 'Bartlett' pears to 60–90 d, so 1-MCP, which almost eliminated the disorders in pears stored for 180 d, could be a useful tool for reducing their incidence. Unfortunately, 1-MCP-treated fruit proved to be recalcitrant in terms of ripening. Softening, ethylene production, respiration rates and ripening were greatly reduced in 1-MCP-treated fruit, even after long periods of storage.

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